Genetic diversity and phylogeography of landlocked seals

Jukka Palo

Department of Ecology and Systematics
Division of Population Biology
P.O.Box 65 (Viikinkaari 1)
FIN-00014 University of Helsinki
Finland

Academic dissertation

To be presented, with permission of the Faculty of Science of the University of Helsinki, for public criticism in the Auditorium 1041 of Viikki Biocenter 2 (Viikinkaari 5) on October 17th 2003 at 12 o'clock noon.

Helsinki 2003

Cover photo:
A Saimaa ringed seal female and her pup
© **Juha Taskinen**

ISBN 952-91-6367-3 (paperback)
ISBN 952-10-1390-7 (PDF)
http://ethesis.helsinki.fi

Edita Prima OY
Helsinki 2003

Supervised by:

Doc. Risto Väinölä
Finnish Museum of Natural History,
University of Helsinki
Helsinki, Finland

Reviewed by:

Prof. Jérôme Goudet
Université de Lausanne,
Lausanne, Switzerland *and*

Prof. Pekka Pamilo
University of Oulu
Oulu, Finland

Examined by:
Prof. A. Rus Hoelzel
University of Durham,
Durham, UK

Author's present address:
Ecological Genetics Research Unit
Department of Ecology and Systematics
Division of Population Biology
P.O.Box 65 (Viikinkaari 1)
FIN-00014 University of Helsinki
Finland

e-mail: jukka.palo@helsinki.fi

Genetic diversity and phylogeography of landlocked seals

Jukka Palo

Original articles in this thesis:

I Palo, J. U. & Väinölä, R. 2003: The enigma of the landlocked Baikal and Caspian seals assessed by mtDNA sequencing. *Submitted manuscript.*

II Palo, J. U., Hyvärinen, H. & Väinölä, R. 2003: Mitochondrial DNA diversity and phylogeography of ringed seals (*Phoca hispida*) from the Arctic Ocean and from Fennoscandian postglacially isolated basins. *Manuscript.*

III Palo, J. U., Mäkinen, H. S., Helle, E., Stenman, O. & Väinölä, R. 2001: Microsatellite variation in ringed seals (*Phoca hispida*): genetic structure and history of the Baltic Sea population. *Heredity* 86: 609-617.

IV Palo, J. U., Hyvärinen, H., Helle, E., Mäkinen, H. S. & Väinölä, R. 2003: Postglacial loss of microsatellite variation in the land-locked Lake Saimaa ringed seal. *Conservation Genetics* 4: 117-128.

1. INTRODUCTION

1.1 The Scope of the Study

Marine organisms inhabit vast and seemingly continuous habitats and are rather inaccessible for direct field observations. However, the advent of molecular markers, i.e. comparisons of nucleic acid or protein molecules, has enabled detailed studies of phylogeny, biogeography, population biology and behaviour of marine species (Bowen et al. 1992, Baker and Palumbi 1996, Graves 1998, LeDuc et al. 1999, Kichler et al. 1999, Hoelzel et al. 1999). In this thesis, modern molecular and analytical tools were employed to elucidate the genetic affinities and history of the landlocked seals inhabiting Lake Baikal and the Caspian Sea, two remote continental basins. Furthermore, the current structure and history of ringed seal (*Phoca hispida*) populations in Fennoscandia and worldwide were explored. Patterns of genetic diversity at various levels of evolutionary divergence were then contrasted with the knowledge of geological history. "The Ice Ages", recurrent Plio-Pleistocene glaciations, are expected to have had an acute impact on the evolutionary history of many North European taxa, including seals.

1.2 "The Ice Age"

The cooling of the Earth's climate during the Cenozoic Era led to the major glaciation periods that started ca. 2.6 million years ago (Mya; Andersen and Borns 1997). Approximately 0.9 Mya the climatic fluctuations became increasingly dramatic, with ice sheets on the circumarctic continents extending to as far south as 40 degrees northern latitude (Andersen and Borns 1997, Thiede et al. 1998). A schematic oxygen isotope curve for the Pliocene and Pleistocene deep-sea sediments demonstrates the cyclical changes in the northern hemisphere climate (Fig. 1). During the glacial maxima, millions of cubic kilometres of evaporated seawater was bound to continental glaciers (Elverhøf et al. 1998).

The advancing ice sheets have repeatedly destroyed boreal terrestrial habitats, restricting species distribution to hospitable areas south of the ice (Hewitt 1996). During the interglacial periods, organisms surviving in these refugia recolonised the previously glaciated areas, at times remarkably rapidly (Hewitt 1996, Taberlet et al. 1998). The recurrent expansions and contractions of the ice sheets have had a two-fold impact on biotic communities. On one hand, by inducing barriers to gene flow, ice sheets may have promoted species diversity through allopatric speciation (Hewitt 1996, Avise et al. 1998, Hewitt 2000, Hewitt 2001). On the other hand, the overall diversity is reduced – the young northern ecosystems are less complex than their old and more stable southern counterparts (Hewitt 2000). In genetic terms, populations inhabiting an area once covered by ice are usu-

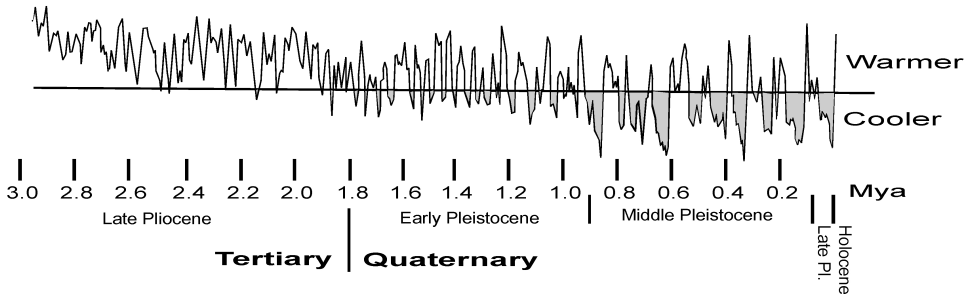

Fig. 1. Quaternary temperature changes reconstructed from oxygen isotope ratio ($^{18}O/^{16}O$) fluctuations in the deep-sea sediment cores from the North Atlantic (reproduced from Andersen and Borns 1997). The curve demonstrates the major steps towards a cooler climate ca. 2.6 Mya, 1.8 Mya and 0.9 Mya.

ally less variable due to the recurrent reductions in population sizes (bottlenecks) associated with the colonisation events (Hewitt 1996). Genetical effects of Pleistocene glaciations have been documented for a number of terrestrial and freshwater organisms (e.g. Sage and Wolff 1986, Väinölä 1994, Merilä et al. 1997, Lunt et al. 1998, Taberlet et al. 1998, Engelbrecht et al. 2000, Hewitt 2000, Comps et al. 2001, Demboski and Cook 2001).

Glacial cycles have profoundly affected marine ecosystems as well (see e.g. Stanley et al. 1996). In the marine environment, the climatic fluctuations were accompanied with changes not only in water temperatures or the extent of ice cover, but also in surface and deep-sea currents (Elverhøf et al. 1998), intensity of coastal upwelling (Berta and Sumich 1999 and references therein) as well as salinity (Chapman et al. 2000). These alterations have changed the productivity of marine ecosystems and further affected the climate (Hewitt 1996, Thiede et al. 1998). The formation of glaciers also caused sea-level regressions that disunited marine basins (Andersen and Borns 1997). Combined, these glaciation-induced factors have had a severe impact on marine organisms of the northern seas but may also have affected species with tropical and temperate distributions (see e.g. Bowen et al. 2001).

1.3 The climatic cycles and the evolution of the phocine seals

At present the northern seas (here taken to include the marine basins between the Northern Atlantic and Bering Strait, as well as the Bering and Okhotsk Seas in the Pacific Ocean) are inhabited by eleven pinniped species, including two eared seal species (Otariidae), the walrus (belonging to a monotypic family Odobeniidae) and eight species classified to the true seal family (Mammalia: Phocidae; Table 1). Together with the landlocked Caspian seal (*Phoca caspica*) and Lake Baikal seal (*Phoca sibirica*), these phocid taxa form the subfamily Phocinae, northern true seals (e.g. Burns and Fay 1970, De Muizon 1982).

The majority of phocines are pagophilic

Table 1. An overview of seal taxonomy (Chapskii 1955a). Subspecies from King (1983).

Order Carnivora					
Family	Otariidae		Eared seals (14 species)		
	Odobeniidae		Walruses (1 species)		
	Phocidae		**True seals**		
Subfamily	Monachinae		Southern true seals (9 species)		
	Phocinae		**Northern true seals**		
		Erignathus	***barbatus***	*barbatus*	Atlantic bearded seal
				nauticus	Pacific bearded seal
		Cystophora	***cristata***		Hooded seal
		Pagophilus	***groenlandicus***		Harp seal
		Histriophoca	***fasciata***		Ribbon seal
Phocina species group		***Halichoerus***	***grypus***		Grey seal
		Phoca	***vitulina***	*vitulina*	E Atlantic harbour seal
				concolor	W Atlantic harbour seal
				richardsi	E Pacific harbour seal
				stejnegeri	W Pacific harbour seal
				mellonae	Ungava seal
		Phoca	***largha***		Larga (spotted) seal
		Phoca (Pusa)	***hispida***	*hispida*	Arctic ringed seal
				ochotensis	Okhotsk Sea ringed seal
				botnica	Baltic ringed seal
				saimensis	Saimaa ringed seal
				ladogensis	Ladoga ringed seal
		Phoca (Pusa)	***caspica***		Caspian seal
		Phoca (Pusa)	***sibirica***		Baikal seal

("ice-loving"), despite differences in their distribution, adaptation to ice cover and prey (Davies 1958). It is supposed that the ancestor of this subfamily lived in the margins of the northern Atlantic during the Middle Miocene ca. 14 Mya (Ray 1976a, Berta and Wyss 1994, but see Koretsky and Holec 2002).

As with most northern taxa, the phocine seals have been subjected to the cyclic changes of the northern hemisphere climate (McLaren 1960, McLaren 1966, Ray 1976b, Perry et al. 1995). During the Pleistocene, expansions of marine ice and concomitant changes in the sea level have allegedly induced the diversification of the Phocina (Chapskii 1955a) species group (Árnason et al. 1995, Hoberg 1995, Árnason et al. 1996, but see Árnason et al. 2000). The formation of continental ice sheets and proglacial lakes has supposedly assisted the colonisation of the Caspian Sea and Lake Baikal by Arctic phocines (King 1983, see also McLaren 1960, Ray 1976b, Sasaki and Numachi 1997). Furthermore, the most recent glacial cycles have had a significant effect on the intraspecific genetic variability, as previously demonstrated for the harbour seal (*Phoca vitulina*; Stanley et al. 1996, Goodman 1998, Burg et al. 1999) and the grey seal (*Halichoerus grypus;* Boskovic et al. 1996).

Despite the general profound effect of the Pleistocene glaciations on the phocines, interspecific differences in severity may exist due to differences in the pagophilic habits of the modern taxa. Whereas the harbour seal and the grey seal inhabit mainly boreal ice-free habitats and whelp predominantly on land (Bonner 1994), the ringed seals breed on ice and are well adapted to arctic conditions. They are also able to maintain entrance and breathing holes in areas covered with fast ice. Consequently, we may expect that the Pleistocene glacial maxima have had a less pronounced effect on the ringed seal populations than on the boreal species.

1.4 The ringed seal – a truly northern northern true seal

The ringed seal is the most abundant and the most arctic of phocine seals (King 1983). It is dependent on stable ice and good snow cover: breeding takes place in subnivean lairs on landfast (preferred) or pack ice (Reeves 1998). As with other seals inhabiting pack ice, ringed seals are believed to be effectively monogamous (Berta and Sumich 1999). Although ringed seals are often considered as philopatric (McLaren 1958), tagging studies have documented migrations of more than 2000 km (Kapel et al. 1998). Seasonal migrations usually follow suitable ice conditions (Kapel et al. 1998, Reeves 1998).

Several million ringed seals inhabit the Arctic Ocean (the nominate subspecies *P. h. hispida*, see Frost and Lowry 1981), occasionally all the way up to the North Pole (Reeves 1998), as well as the North Atlantic, North Pacific and Okhotsk Sea (Fig. 2a; King 1983). Being ubiquitous and relatively easy to hunt, ringed seals are of vital importance for two other Arctic mammals: the polar bear (*Ursus maritimus*) and man (*Homo sapiens*). The ringed seal continues to hold high economic value for the Inuit communities (Reeves 1998).

In addition to the main Arctic population, ringed seals inhabit the Baltic Sea, the White Sea, Lake Saimaa and Lake Ladoga in Fennoscandia (Fig. 2b). These marginal populations were established at the end of the last (Weichselian) glaciation phase. Atlantic ringed seals invaded the Baltic basin during the deglaciation ca. 11 500 years ago and were cut off from the main Arctic stock ca. 10 900 years ago, when the marine connection between the North Sea and the Baltic (Yoldia) Sea temporarily closed (Forstén and Alhonen 1975, Donner 1995; Fig. 3). Soon after the initial isolation, part of the Baltic population became trapped into several Fennoscandian lakes, newly emerging due to rapid isostatic land uplift (Müller-Wille 1969, Donner 1995). Today, these landlocked seals survive in Lake Saimaa and Lake Ladoga. They are now considered as subspecies *P. h. saimensis* and *P. h. ladogensis* with diagnostic morphological differences between them (Hyvärinen and Nieminen 1990).

During the last 10 000 years (10 ky, the Holocene), the main Arctic population has probably remained large, today comprising perhaps up to 7 million individuals (Reeves 1998). The

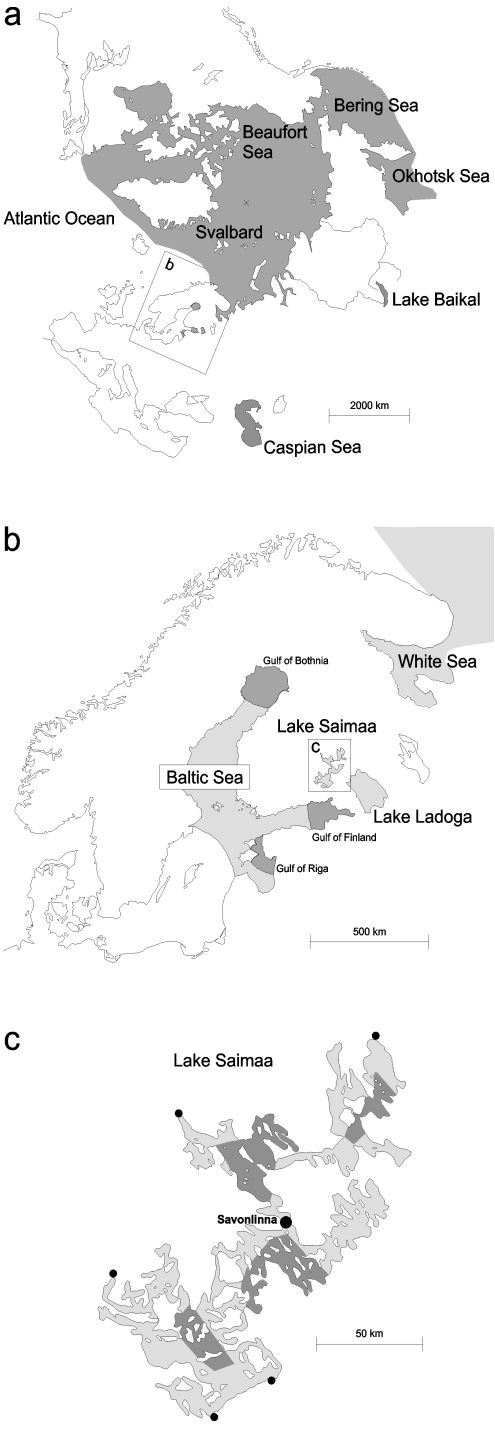

Fig. 2. The distribution of the ringed seal (grey) a: worldwide, b: in the four Fennoscandian postglacial basins and c: within Lake Saimaa. In b and c, primary breeding areas are indicated with darker shading.

inferred historical population size of the Baltic population has also been large, ca. 100 000– 300 000 (Durant and Harwood 1986, Hårding and Härkönen 1999, Kokko et al. 1999). An estimated 10 000 - 20 000 seals have inhabited Lake Ladoga in the beginning of the 20th century (Sipilä and Hyvärinen 1998). Meanwhile, the Saimaa ringed seal population has probably never been particularly large; estimates for historical population sizes range from 2 500 (Hyvärinen and Sipilä 1992) to less than 1 000 (Kokko et al. 1999). These postglacial relict populations have all experienced a strong decline in the 20th century (Sipilä et al. 1990, Härkönen et al. 1998, Sipilä et al. 1996).

1.5 The study

By assessing patterns of contemporary genetic diversity, I have aimed to elucidate the phylogenetic affinities of the landlocked Caspian and Baikal seals, and the Quaternary history of the ringed seal populations in the Arctic Ocean and in the Fennoscandian marginal postglacial basins. More specifically, the questions in the four papers (*I–IV*) included were:

1.5.1 The origin of the landlocked Baikal seal and Caspian seal (*I*).

The enigmatic presence of seals in two old Eurasian basins, Lake Baikal and the Caspian Sea (Fig. 2a), has drawn much attention and generated a variety of hypotheses about the origin and genetic affinities of these seals (e.g. Chapskii 1955b, Davies 1958, McLaren 1960, Sasaki and Numachi 1997). In most cases, these landlocked species are considered to descend from the ringed seal; the three species comprise the (sub)genus *Pusa* (Scheffer 1958). Ray (1976b) and Koretsky (2001; see also Árnason et al. 1995) suggested that *Pusa* emerged in the Paratethys, a brackish inland sea covering large parts of southeast Europe and Asia Minor ca. 15–5.5 Mya (e.g. Grigorescu 1976). In contrast, McLaren (1960), along with several other authors, argued that the ancestor of the *Pusa* lived in the North Atlantic or Arctic Ocean. The suggested times for the continental invasion have

varied from the Late Miocene ca. 6 Mya (McLaren 1960) to the Middle Pleistocene ca. 300 kya (Davies 1958, Sasaki and Numachi 1997). Hypotheses explaining the peculiar landlocked distribution and the timescale of the continental invasions were evaluated here by constructing a mitochondrial gene phylogeny for Baikal and Caspian seals and seven other phocine taxa.

1.5.2 Quaternary population history of the Arctic ringed seals (II, III)

The actual number as well as the stock identity of the large Arctic ringed seal population, classified into one subspecies *P. hispida hispida* (Frost and Lowry 1981), is largely unknown (Reeves 1998).

Here, genetic diversity of the Arctic ringed seals from Alaska (the Bering and Beaufort Seas) and the Svalbard archipelago was investigated to examine the Quaternary history of the main ringed seal population. The population history was inferred from patterns of intraspecific genetic diversity and through interspecific comparisons. As no drastic demographic changes are believed to have taken place in the Arctic ringed seals since the last deglaciation, the genetic diversity of the main Arctic stock also provides a reference point against which the diversities of the postglacial populations can be compared.

1.5.3 Holocene history of the ringed seals from the Baltic and White Seas (II, III).

It has been suggested that the Baltic ringed seals have remained isolated from the Arctic population during the Holocene, mainly due to distance and inhospitable (ice-free) areas along the Norwegian coast, in the North Sea and South Baltic (Müller-Wille 1969, Forstén and Alhonen 1975). Although large throughout most of its 10 ky history, the size of the Baltic population declined severely in the 19th and 20th centuries due to human actions. In the mid 1990's, only an estimated 5 500 (hauled-out) ringed seals remained in three geographically separate main breeding areas in the Gulf of Bothnia (ca. 4000 ind.), Gulf of Finland (ca. 150 ind.) and Gulf of Riga (ca. 1 500 ind., Fig 2b; Härkönen et al. 1998).

Microsatellite and mtDNA diversity of the Baltic ringed seals were contrasted with the Arctic diversity patterns to infer Holocene population history, e.g. effective population size and gene flow between the Baltic and Arctic ringed seals. Differentiation between two Baltic breeding areas (the Gulf of Finland and Gulf of Bothnia) was also assessed. In addition, mtDNA diversity in a sample of eight specimens from the White Sea was examined to estimate the genetic diversity and differentiation of this largely unknown postglacial population.

1.5.4 Genetic diversity and population structure of the landlocked ringed seals (II, IV).

The two lacustrine daughters of the Baltic ringed seals in Lake Saimaa and Ladoga have been landlocked for roughly 9 500 and 9 000 years, respectively (Forstén and Alhonen 1975, Donner 1995, Björck and Sandgren 1987). Lake Saimaa in SE Finland differs drastically from the open continuous marine seal habitat: it is a shallow (mean depth 12 m) labyrinthine lake network dotted with some 14 000 islands (Kuusisto 1999). Human actions brought the Saimaa

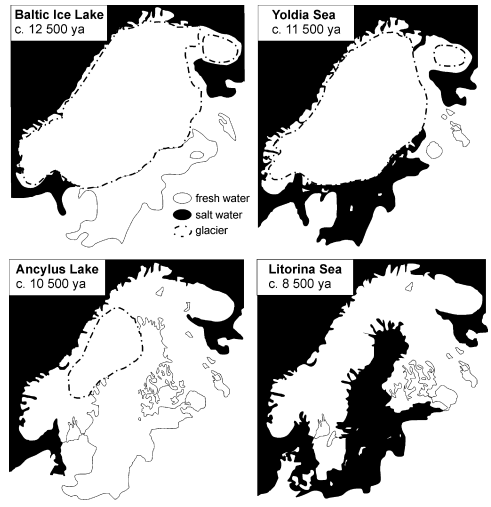

Fig. 3. The geological history of the Baltic Sea basin in the Holocene. Arctic ringed seals, which followed the retracting ice margin invaded the Baltic basin most likely during the Yoldia phase c. 10 900 years ago (reproduced with permission from Eronen et al. 2001).

ringed seal to the edge of extinction in the 20th century. At the beginning of the 1980's, only 100 seals remained in four separate breeding areas (Fig. 2c; Sipilä et al. 1990). Since then, there has been a gradual increase – in 2001 the population numbered ca. 220 seals, mainly in the central parts of the lake (Sipilä and Koskela 2001). Ranta et al. (1996) and Kokko et al. (1998) have shown that the Saimaa ringed seal is under a constant threat of extinction due to demographic stochasticity, possibly enhanced by population fragmentation in the complex lake system.

The (relative) genetic risks for the survival of the Saimaa ringed seal were evaluated based on the inferred post-glacial increase of inbreeding. The Saimaa ringed seal provides a good model population for the study of genetic effects of postglacial isolation because: a) the amount of genetic variation in the founder population can be estimated by reference to the marine ringed seals of the Baltic Sea and Arctic Ocean and b) the time of isolation is known from the geological record of Fennoscandian lake history.

In addition, the genetic effects of the population fragmentation within the lake were roughly evaluated by inferring the differentiation between the northern and southern parts of Lake Saimaa from DNA microsatellite data. Implications of the genetic results for the conservation of the Lake Saimaa and Baltic ringed seals are also discussed.

2. MATERIALS AND METHODS

2.1 Samples

Nine of the ten phocine species (excluding the harp seal, *Pagophilus groenlandicus*) were studied for assessment of the phylogenetic affinities of the Baikal and Caspian seal. To reconstruct the intraspecific phylogeography of the ringed seal, more than 350 samples representing the main Arctic population (Alaska and Svalbard) and four isolated Fennoscandian relict populations (Baltic Sea, White Sea, Lake Saimaa and Lake Ladoga) were obtained. The muscle and liver tissue used as a source of DNA were from samples collected for a variety of scientific purposes, e.g. environmental toxin monitoring. The Saimaa ringed seals were not disturbed by intrusive sample collection – the material came from seals found dead and returned to the University of Joensuu from 1980 onwards. The tissue samples were preserved frozen or in ethanol.

2.2 Molecular markers – an overview

Here, a general description of the methods used in the study is presented; laboratory procedures and analytical methods are specified in detail in the original papers.

Contemporary genetic diversity has been shaped by the ecology and history of the species or population in question. Consequently, the genomes carry information regarding these events (e.g. mating system, vicariance events or population size changes), which can be inferred from patterns of genetic diversity at molecular marker loci (Avise 1994, Hillis et al. 1996). However, due to e.g. selection, the molecular genealogies do not necessarily recapitulate organismal history and therefore reliable inferences of organismal history require data from multiple unlinked marker loci (Nichols 2001, Ballard et al. 2002, Hewitt 2001).

Direct DNA-level comparisons, especially of the noncoding parts of the genome, became widely feasible by the advent of PCR (Polymerase Chain Reaction) technology in the late 1980's. Since this "PCR-revolution" two marker classes, nuclear microsatellites and mitochondrial DNA (mtDNA) sequences, have dominated phylogenetic and population genetic assessments (Avise 2000).

2.2.1 Mitochondrial DNA sequences

In phylogenetic inference, mitochondrial DNA markers have several advantages over nuclear sequences. The largely clonal, maternal inheri-

tance and lack of recombination (see Macaulay et al. 1999) enable the recovery of intraspecific matrilineal genealogies. Due to considerable variation in the evolutionary rates between different segments (see Lopez et al. 1997, Pesole et al. 1999), mtDNA sequences can be informative across a large span of evolutionary time (e.g. Hillis et al. 1996, Avise 2000).

Phylogenetic patterns are often geographically restricted (e.g. Hewitt 2001). Phylogeography investigates the geographical distribution of genealogical lineages (Avise et al. 1987); it yields information on current gene flow as well as past evolutionary history (Fedorov et al. 1996), adding an evolutionary perspective to biogeographic analyses (Smouse 1998).

The formulation of the coalescent theory (Hudson 1990) connected phylogeny and demography mathematically, thus allowing inference of population history from genetic data. Yet, these inferences, especially from the hypervariable regions of the mtDNA genome, can be impaired by the complex dynamics of molecular evolution (e.g. Salas et al. 2000, Arbogast et al. 2002). Although often critical for the accuracy of inferences of population history, the mode and rate at which the new allelic variants arise at the marker loci is not always well understood (see e.g. Driscoll et al. 2002).

Sequence data were obtained from three mitochondrial protein coding genes cytochrome *b* (Cyt *b*, 1140 bp), cytochrome oxidase I (COI, 1545 bp) and cytochrome oxidase II (COII, 684 bp). Based on these data, the affinities and timescale of the continental invasions of the Caspian and Lake Baikal seals were inferred.

Furthermore, sequence variation in 416 bp of the noncoding mitochondrial control region (CR) was assessed to evaluate the Quaternary history of ringed seal populations in the Arctic and several Fennoscandian populations.

2.2.2 Microsatellites

Microsatellites are highly polymorphic stretches of tandemly repeated 1-5 bp DNA elements (e.g. …ACACACA...), often highly variable in the repeat number. They have proven informative in genetic studies from the individual ("genetic tagging", paternity analyses, assignment tests) to population levels (estimation of gene flow between populations, demographic history or effective population size). Their utilisation in ecological studies has been covered in reviews by Queller et al. (1993), Jarne and Lagoda (1996), Estoup and Angers (1998), Goldstein and Schlötterer (1998) and Balloux and Lugon-Moulin (2002). Increased resolution in population genetic analyses can be acquired by including data on both frequencies and the genealogy of the alleles. Analytical tools based on maximum likelihood, coalescent and Bayesian methods enable the inference of more detailed information of, for example, evolutionary parameters (e.g. mutation and migration rates), effective population size or coalescence (reviewed in Luikart and England 1999).

Numerous microsatellites have been designed for the study of pinniped populations (e.g. Allen et al. 1995, Goodman 1997, Andersen et al. 1998, Hoelzel et al. 1999, Kretzmann et al. 2001). Many of the primers successfully amplify microsatellite loci in several seal species (Gemmell et al. 1997). Using eight primer pairs originally designed for the harbour seal (*SGPV-*; Allen et al. 1995) and the grey seal (*Hg-*; Goodman 1997), genetic diversity in the Arctic, Baltic and Lake Saimaa populations were assessed in order to evaluate the history of these populations.

3. RESULTS AND DISCUSSION

3.1. The origin of Baikal and Caspian seals: evidence for Pliocene Arctic invasion of the two Asiatic continental basins *(I)*

In the total data set of 3 369 bp of protein coding mitochondrial sequence from nine phocine taxa 719 variable sites were observed. The phylogenetic analyses showed that the Baikal and Caspian seal lineages group closely together with the grey, ringed, harbour and larga (*Phoca largha*) seal mtDNA lineages (Phocina-group; Chapskii 1955a; see Table 1). Resolution among these Phocina lineages was poor, as

evidenced by the unresolved trichotomy and the low branch support in the phylogenetic trees (Fig. 4).

While monophyly of the *Pusa* lineages was not conclusively ruled out by the mtDNA data there appears to be no particular affinity between the ringed, Baikal and Caspian seals. Rather, in the trees the Caspian seal clustered together with the grey seal, although the support also for this association is weak.

A likelihood ratio test (Kishino and Hasegawa 1989, Shimodaira and Hasegawa 1999) revealed significant interlineage variation in the mtDNA divergence rates among the phocine taxa. Rate variation between lineages confounds reliable estimation of divergence time assuming a molecular clock (Arbogast et al. 2002), but this may be at least partially circumvented by using the non–parametric rate smoothing (NPRS) method of Sanderson (1997). Using this method, the phocine divergence times were estimated as proportions of the cat (*Felis catus*) - dog (*Canis familiaris*) divergence from a ML-tree that included these two additional taxa as well as outgroup sequences from the finback whale (*Balaenoptera physalus*) and hippopotamus (*Hippopotamus amphibius*; see *I*).

The NPRS-method suggests that the basal phocine divergence constitutes 29.0% of the cat-dog divergence. Based on palaeontological reference from a number of vertebrate taxa and multigene data, the absolute time of the cat-dog divergence has been dated to 55 (50 – 60) Mya (Springer et al. 2003). Assuming this calibration, the root of the phocine mtDNA linegaes would be dated to 0.29 x 55 = 16.0 (14.5 – 17.4) My. Similarly, the root of the Phocina group mtDNA lineages make 5.2% of the cat–dog divergence, and would be placed at 2.9 (2.6 – 3.1) My.

However, as species divergence involves sorting of lineages already present in the ancestral population, the molecular divergence necessarily predates species divergence (Edwards and Beerli 2000). Assuming that the intraspecific diversity (i.e. long term effective population size) in the ancestral population was similar to that of the modern taxa (esp. *P. hispida*) we can guesstimate the magnitude of difference between the molecular and species divergence. The essential feature of the mtDNA variation in the ubiquitous Arctic ringed seal (II) is the presence of numerous old lineages, which trace back to up to ca. 1.0 Mya (see below). If the genetic constitution of the ancestral population was similar, the molecular data would overestimate the species divergence maximally by this age, implying that radiation of the Phocina group (species divergence) would have taken place ca. 1.9 Mya. This estimate coincides with the most recent split among the Phocina mtDNA lineages – the divergence between *H. grypus* and *P. caspi-*

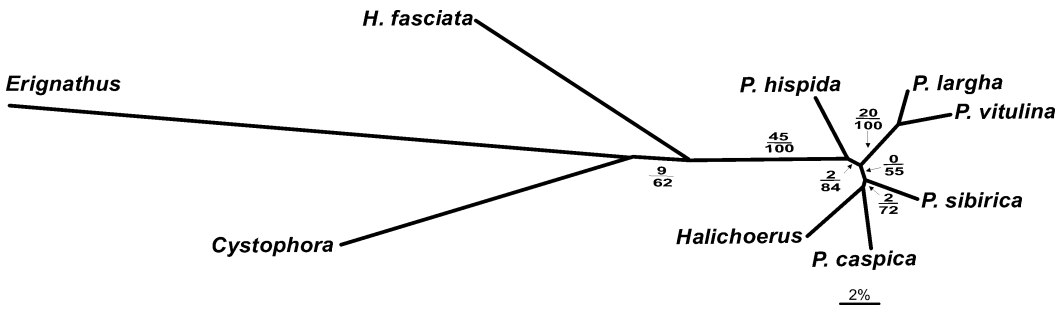

Fig. 4. A maximum parsimony / Neighbour-joining phylogeny of the nine phocine taxa. The NJ-tree was constructed assuming gamma-corrected HKY85 substitution model (Hasegawa et al. 1985) with shape parameter $\alpha = 0.154$. The numbers indicate Bremer support (above; Bremer 1994) and bootstrap values (below the lines) for the given branch. The five Phocina lineages appear equally closely related to each other, and may have diverged some 2.9 Mya.

ca dated at 2.0 My using the NPRS method. Assuming a star phylogeny for the Phocina lineages, the latter could also be taken as a reasonable estimate for the species divergence date.

In contradiction to earlier hypotheses of a Paratethyan origin for the subgenus *Pusa* (Ray 1976b, Koretsky 2001), or tribe Phocini (Árnason et al. 1995), the relatively concurrent radiation among Phocina and the oceanic distribution of most of the modern taxa suggest that the diversification of this group took place in the northern oceans. As opposed to scenarios involving an origin in the temperate Paratethyan Sea, the Arctic origin hypothesis would plausibly explain the emergence of the white natal hair and the perpetuation of the ice breeding habit in the Phocini (*cf.* Perry et al. 1995).

It therefore appears likely that Lake Baikal and the Caspian Sea were invaded by marine ancestors from the north, as proposed by several authors (Chapskii 1955b, Segerstråle 1957, Davies 1958, McLaren 1960). This, as such, would be consistent with the notion of *P. caspica* as part of the "Arctic element" of the Caspian Sea fauna, which also includes several coldwater invertebrates and fish (see Segerstråle 1957). However, the authors above suggested that the continental invasion by seals took place either in the Tertiary (> 5 Mya; McLaren 1960) or Middle Pleistocene, facilitated by the creation of large ice-dammed lakes in central Siberia ca. 300 kya (Pirozhnikov 1937, Chapskii 1955b, Davies 1958). In addition, Zubakov (2001) has proposed that the entry of seals was associated with a major Pliocene Caspian transgression ca. 3.4 Mya. In contrast to these timescale suggestions, the mtDNA sequence data rather point to invasions during the Late Pliocene roughly 2 Mya, i.e. after the Caspian transgression, but prior to the major continental glaciations that started ca. 0.9 Mya. The paleohydrography allowing the access to the landlocked basins thus remains enigmatic.

3.2. Genetic diversity in the Arctic ringed seals indicate weak impact of Pleistocene glaciations (*II*, *III*)

Nuclear and mitochondrial markers confirm high levels of genetic diversity in the Arctic ringed seals. A unique mtDNA control region haplotype was detected in all forty-six individuals from Alaska (the Beaufort and Bering Seas) and the Svalbard archipelago. Nucleotide diversity was high (Table 2) and the phylogenetic pattern involves several deep lineages (Fig. 5). As with the mtDNA diversity, microsatellite heterozygosity was high (Table 2) among the 39 Svalbard specimens studied. Analysis of molecular variance (AMOVA) showed no significant geographic structuring of mtDNA variation between Alaska and Svalbard samples (Φ_{ST} = 0.003, P = 0.351).

The distribution of pairwise differences (mismatch distribution, see *II*) of the Arctic control region haplotypes was slightly bimodal, with the prominent peak at around 20 observed differences (or at around 27 substitutions, estimated assuming gamma-corrected Kimura 2-parameter substitution model; Kimura 1980, Schneider and Excoffier 1999). The observed distribution could not be distinguished from the unimodal distribution expected under a stepwise population expansion. According to this model the population suddenly expanded from N_0 to N_1, $\tau = 2ut$ mutational events ago (where u is the number of mutations per haplotype and t the number of generations; Rogers and Harpending 1992).

In the Cyt *b* data, the main intraspecific mtDNA lineages observed in *P. hispida* constitute ca. 34% of the average interspecific divergence among five Phocina taxa (*P. hispida*, *P. sibirica*, *P. caspica*, *P. vitulina*, *H. grypus*; synonymous changes, estimated with the PBL-method; Pamilo and Bianchi 1993 Li 1993). If the mtDNA lineages of these taxa diverged ca. 2.9 Mya (above), this would suggest that the intraspecific mtDNA divergence in *P. hispida* would have originated some 1.0 Mya, which, assuming an 11-year generation interval, would imply a rate of $u = 1.8 \cdot 10^{-4}$ substitutions haplo-

type^{-1} generation^{-1} in the control region (*III*, *II*). Estimated from the mismatch distribution, the demographic event producing the main peak in the Arctic mtDNA sequences took place $\tau = 2ut$ = 27.3 (20.4 – 33.6) mutational units ago, which, assuming the above u, would trace back to 0.84 (0.63 – 1.02) million years ago.

Assuming the demographic model of Rogers and Harpending (1992), the control region diversity suggests a very large long term post-expansion effective size for the Arctic seals. The size scaled with a mutation rate was $\theta = 2N_e u$ = 127, which, assuming the rate above, would indicate effective female population size of ca. $N_e = 3.5 \cdot 10^5$. From the microsatellite diversity in the Svalbard ringed seals we get a somewhat smaller estimate for female effective size of $N_e \approx 1.6 \cdot 10^4 – 1.6 \cdot 10^5$. This inference assumes microsatellite mutation rates between 10^{-4} and 10^{-5}, mutation-drift equilibrium and equal sex ratios.

In the Arctic ringed seals the intraspecific genetic diversity was very high and traces back to perhaps up to ca. 1.0 My, which appears rather exceptional intrapopulational divergence for an Arctic species. The observed diversity pattern is compatible with a demographic and range expansion (see Ray et al. 2003) that coincided with the start of the most dramatic Pleistocene glaciation phases. This suggests that the Arctic ringed seals have been able to maintain large populations size and high migration rates throughout the Pleistocene. As the ringed seals are well adapted to arctic conditions, they have probably been able to effectively exploit the increase of suitable breeding habitat during the glacial periods. This is further supported by interspecific comparisons: significantly lower mtDNA variation levels has been reported from the harbour seal, a common boreal phocine largely restricted to ice-free areas of the Atlantic and Pacific Oceans (Bonner 1994). In agreement, microsatellite diversity (H = 0.82) suggests that the long-term effective population size of the ringed seal have been 6.5 times that of the harbour seal, and 1.5 times that of the grey seal (assuming mutation-drift equilibrium and a single-step mutation model, e.g. Nei 1987).

3.3. Genetic diversity in the Fenno-scandian postglacial populations (*II-IV*)

3.3.1 Conflicting results from different markers

In general, the postglacially established Fennoscandian populations showed successively lower genetic diversities as compared to the Arctic ringed seals (Table 2), although the Baltic Sea (and Lake Ladoga) population still retain high mtDNA haplotype (*II*) and microsatellite (*III*) diversity. The overall microsatellite allele frequency differentiation between the Arctic and the Baltic was low (F_{ST} = 0.023). Within the Baltic population, no signs of geographical segregation of microsatellite or mtDNA diversity between Baltic Sea breeding areas (Gulf of Finland / Gulf of Bothnia) were detected.

The mtDNA nucleotide diversity in all the Fennoscandian populations is clearly reduced, however. The mtDNA variability is organized in five deeply rooted lineages (A-E, separated by 13-28 differences) with relatively shallow divergence within each intralineage haplogroup (less or equal to 6 differences, Fig. 5c). Four of these lineages were detected in the Baltic Sea, three in Lake Ladoga and one in both Lake Saimaa and the White Sea. Except in lineage A, Fennoscandian haplotypes intermix with Arctic haplotypes within lineages and four of the lineages

Table 2. Estimates of genetic diversity in the Arctic and Fennoscandian ringed seal populations.

MtDNA control region (416 bp)

	N	*Haplotype div. (H)*	*Nucleotide div. (π_n)*	*Relative π_n*
Arctic	46	1.00 ± 0.00	0.069 ± 0.034	100%
Baltic	38	0.98 ± 0.01	0.055 ± 0.027	80%
White Sea	34	0.89 ± 0.11	0.007 ± 0.005	10%
Saimaa	25	0.62 ± 0.07	0.009 ± 0.005	13%
Ladoga	8	0.95 ± 0.02	0.023 ± 0.012	33%

Microsatellites (8 loci)

	N	*Alleles (A)*	*Relative A*	*Gene diversity (H_e)*	*Relative H_e*
Svalbard	39	99	100%	0.82	100%
Baltic	149	93	94%	0.80	98%
Saimaa	80	16	16%	0.25	31%

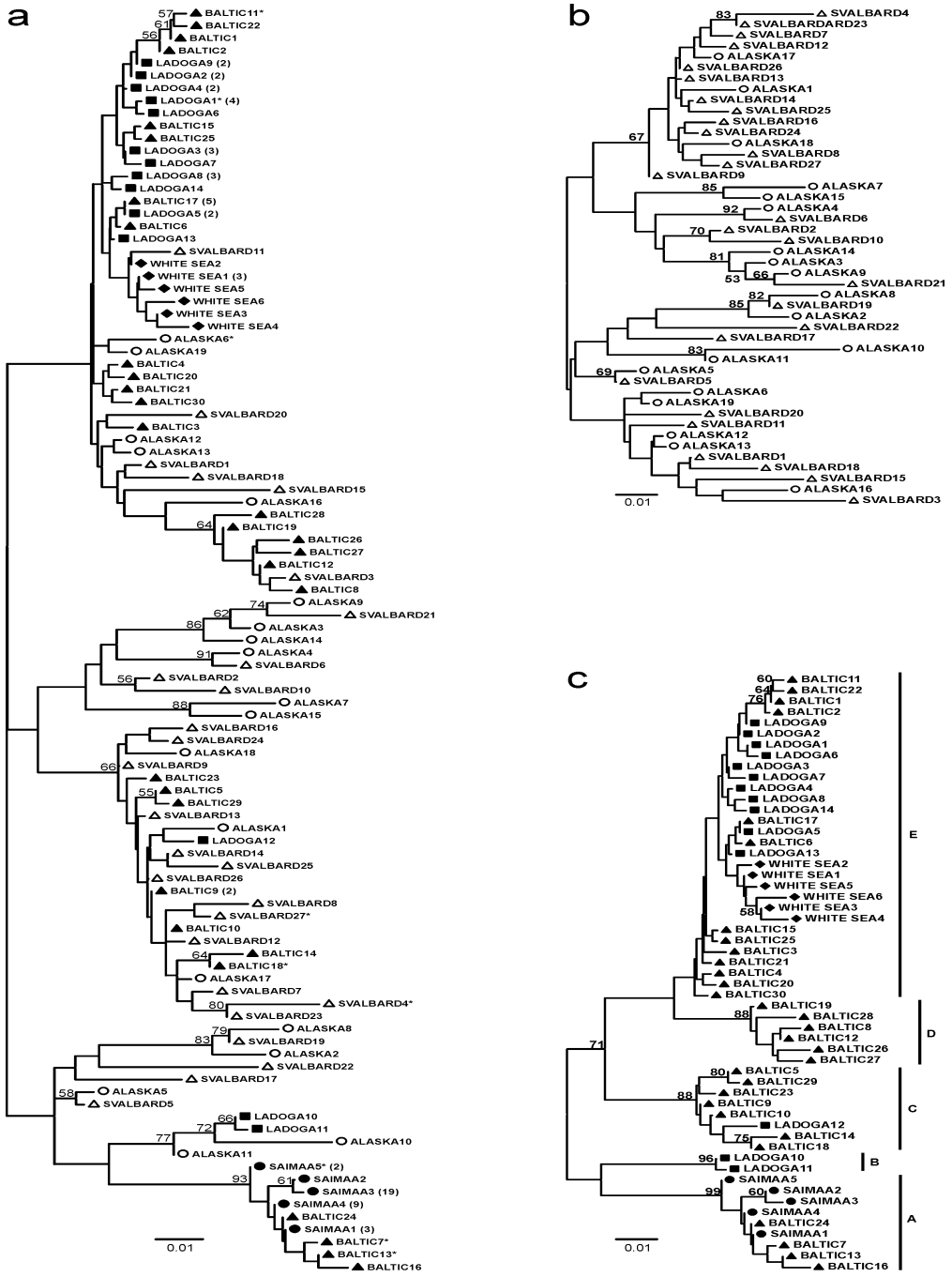

Fig. 5. MtDNA control region diversity in the ringed seals visualised by NJ-trees; haplotypes from a) all, b) Arctic and d) postglacial ringed seal populations. The mutation model assumed in the analysis was Tamura-Nei (1993) with gamma correction for rate heterogeneity between sites (α=0.13). The numbers denote bootstrap support values. The Fennoscandian mtDNA variability is organised in five distinct lineages A-E.

(A, C-E) were shared between two or more Fennoscandian basins (Fig. 5a).

To explain the distinctive patterns of genetic diversity, two alternative scenarios of population history must be considered, neither of which is firmly supported by the data. In what follows, I will evaluate these "post-glacial" and "pre-Holocene" hypotheses.

The geographic segregation of the shallow mtDNA clusters readily suggests a ("postglacial") scenario where a bottleneck associated with the colonisation of the Baltic basin initially reduced the number of ancestral Arctic lineages to five or so. Further losses of the ancestral mtDNA lineages then took place in each basin during the postglacial time. Within the remaining lineages, mutations accumulated postglacially generating the intralineage variation. This hypothesis, however, suffers from several problems.

The scenario would require a severe Baltic bottleneck reducing the number of Atlantic haplotypes soon after the initial colonisation before entrapment of the lake populations, and subsequent isolation of the Baltic population from the Arctic seals. This is at odds with high microsatellite diversity in the Baltic and recurrent gene flow between Arctic and Baltic populations, also inferred from the microsatellite data (*III*). In addition, the hypothesis would demand a monophyly of haplotypes from each postglacial basin, which is refuted by maximum parsimony analyses of two data subsets (*II*).

In an alternative "pre-Holocene" scenario, even the intralineage CR variation would predate the colonisation of the Baltic basin. Using the Cyt *b* – based CR substitution rate estimates (above), the divergence would have started ca. 180 kya and the putative reduction of lineages would have predated that event. This would be plausibly explained by demographic and/or range expansion during the Saalean glaciation (*cf.* results from the Arctic population above).

The pre-Holocene hypothesis also has several counterarguments. It seems to require that the Baltic population derived from an unidentified ancestral stock in the North Atlantic, effectively isolated from the here assessed Svalbard-Alaska ringed seals throughout much of the Weichselian. The weak microsatellite divergence between the Arctic and Baltic populations is also in sharp contrast with this scenario. A higher divergence in mitochondrial relative to nuclear markers could result from male-biased gene flow in the ringed seal. Gene flow in several mammal species is highly asymmetric, with females being more philopatric than males (see e.g. Greenwood 1980, O´Corry Crowe et al. 1997, Burg et al. 1999, Nyakaana and Arctander 1999). However, tagging studies of ringed seals have failed to identify any strong male bias in ringed seal dispersal patterns (Kapel et al. 1998).

Non-monophyly of the Fennoscandian clusters with respect to the Arctic stock is a problem also for this hypothesis, but it could be partly overcome by assuming that the Svalbard population represents a merger of the hypothetical Atlantic and a truly Arctic stock. Roughly half of the Svalbard haplotypes cluster in lineage C, and the average divergence among these haplotypes is in the same order as intralineage variation in the postglacial populations (ca. 2%, Fig. 5a).

Particularly problematic for the pre-Holocene hypothesis is the segregation of the five Fennoscandian lineages among the four basins. If we assume that the mtDNA variation in the Baltic population has remained similar through the Holocene, the retention of a number of haplotypes belonging to only one lineage in Saimaa and White Sea is improbable. The survival of multiple colonising haplotypes of lineage A in Lake Saimaa is also inconsistent with inferences of post-glacial effective population size (*IV*, see below).

The likelihoods of these two alternative hypotheses depend critically on the mutation frequencies at the marker loci. The pre-Holocene hypothesis would be compatible with the Cyt *b*-based rate estimated above, and also with rates generally suggested for mammalian mitochondrial coding regions (e.g. Pesole et al. 1999). In contrast, the postglacial hypothesis would require about twenty times higher CR substitution rate to generate the control region variation

detected in the Fennoscandian populations during the Holocene, which appears exceptional. Nevertheless, some control region sites are known to evolve remarkably rapidly (Heyer et al. 2001). We may suspect that the used mutation models (gamma-corrected TrN93- and K2P-models) are not able to account for the suspected extreme rate heterogeneity in the control region, thus leading us to overestimate the age of the recent divergences (Arbogast et al. 2002). A high mtDNA mutation rate also seems to accord well with the intralacustrine coalescence history of the mtDNA molecules (see below), but would lead to twenty times lower effective population size estimates, compatible with the Arctic N_es inferred from microsatellites only if also the microsatellite mutation frequencies were high.

Thus counterarguments to both hypotheses remain. The Atlantic ancestors of the Fennoscandian ringed seal populations appear to have a complex history. Additional sampling, especially from the western Atlantic, and analyses with other DNA markers are required to further elucidate the past. However, for the remaining discussion, the postglacial hypothesis is considered as more plausible.

3.3.2. The landlocked populations: postglacial loss (and gain) of genetic diversity (II, IV)

The Saimaa ringed seals have 68% lower microsatellite and 87% lower mtDNA CR nucleotide diversity than the marine reference population. Assuming mutation rates of around 10^{-4}, the actual loss of microsatellite diversity would be $F \sim 0.74$. A reduction of this magnitude would correspond to an average effective size of ~320 seals during the 860 generation-long lacustrine isolation (estimated as $N_e = -0.5t/\ln(1-F)$; e.g. Hartl and Clark 1997). The coalescent based maximum likelihood method of Berthier et al. (2002; assuming no mutation) gave a slightly higher estimate $N_e = 451$ (95% confidence interval 296 – 659).

The reduction of genetic variability (as measured from the microsatellite loci) in the Saimaa ringed seals is similar to those reported from several other endangered mammals, e.g. the southern elephant seal *M. leonina* (Hoelzel et al. 1999) or Ethiopian wolf *Canis simensis* (Gottelli et al. 1994). In the Saimaa ringed seal the ratio of the number of microsatellite alleles to the allele size range, expected to decrease when alleles are randomly lost in a bottlenecked population, was the smallest reported thus far, $M = 0.57$ (*IV*, Garza and Williamson 2001).

Given that the N_e / N ratios in mammals can be as low as 0.1 (Frankham 1995), the inferred long-term effective population size of ca. 350 specimens for the Saimaa ringed seals is marginally compatible with the proposed census size of 2 500 in the past (Hyvärinen and Sipilä 1992). If this N_e estimate roughly holds, it lends additional support for the retention of only one ancestral mtDNA lineage in Lake Saimaa, making the postglacial hypothesis of mtDNA diversity in the Fennoscandian populations (above) more plausible. Simulation studies have shown that isolated daughter populations formed randomly from a large, well-mixed ancestral population attain monophyly with high probability in $4N_{e(f)}$ generations (Avise 2000). Consequently, assuming equal sex ratios and stable population sizes, the Saimaa ringed seal would have attained monophyly in 700 generations or 7 700 years, i.e. within the time span of their postglacial isolation. The microsatellite diversity would thus concur with the mtDNA results only if we assume that the five Lake Saimaa mtDNA haplotypes arose during the postglacial isolation. However, as mentioned above, this would require abnormally high substitution rates in the control region.

Although the Saimaa ringed seal population now appears geographically subdivided in the complex lake system (Fig. 2c), microsatellite markers suggest that the Saimaa ringed seal population has in the past acted as an effectively single genetic unit ($F_{ST} = 0.02$). However, due to the limited sample sizes, we could not reliably assess the peripheral areas and the test remains rather crude.

Unlike Lake Saimaa, Lake Ladoga is a deep (mean 51 m, max. 225 m) and more continuous environment (Kunnasranta 2001). The Lake Ladoga ringed seal population is in many aspects an intermediate between the Baltic and

the Lake Saimaa population. It has been isolated like the Saimaa ringed seals, but the size of this relict population has plausibly been several times larger, perhaps 10 000 – 20 000 individuals.

Three deep mtDNA lineages are retained in the Lake Ladoga sample. As no microsatellite data exist, we get a rough estimate of $N_{e(f)} \sim$ 1 050 for the Lake Ladoga population by multiplying the Lake Saimaa estimate by six (ratio of census size estimates). Under these assumptions, we would expect two ancestral lineages to remain in the lake; $N_{e(f)} \sim 1\,600$, marginally compatible with the census estimates (assuming $N_e / N = 0.1$), would have ensured retention of three ancestral lineages (see p. 708 in Emerson et al. 2001). As with Lake Saimaa, this expectation would also be consistent with the postglacial mtDNA diversity hypothesis. A significant postglacial expansion would be required to produce the mismatch wave peaking at around four differences in the Lake Ladoga mtDNA data.

3.4. Looking ahead: conservation aspects

Despite some potential threats like global warming and pollution, the main Arctic ringed seal population is considered secure (Reeves 1998). Conversely, the postglacial landlocked populations are constantly threatened by environmental and demographic stochasticity (Kokko et al. 1997, Kokko et al. 1998). In particular, the Saimaa ringed seal has been on the verge of extinction due to anthropogenic factors (e.g. Sipilä and Hyvärinen 1998) since the mid 20th century. Showing a high degree of site-fidelity (Koskela et al. 2002; but see *IV*), the Saimaa ringed seals are readily affected by changes in their environment, e.g. by an increasing number of leisure apartments as well as recreational boat and snowmobile traffic (Kunnasranta 2001).

With the advent of novel molecular methods genetic studies of endangered taxa have become widespread (Avise 1995). Nevertheless, the relative importance of genetic data for conservation has been a highly contentious issue. For example, Lande (1988) and Caro and Laurenson (1994) have argued that ecological and demographical data are more important for making conservation decisions. However, as noted by Avise (1996), these authors associate genetic analysis to a mere heterozygosity assessment. Information deriving from population genetic and phylogenetic analyses of DNA marker data can yield information on e.g. systematics, genetic diversity, spatial patterns, gene flow, demographic history, kinship and mating systems, all invaluable for conservation considerations (Avise 1996, Hedrick 2001).

Population genetic data have been widely used to identify units relevant for conservation, especially in the marine realm where species or population boundaries are not obvious (Avise 1998). Below the species-level, DNA markers have been used to identify intraspecific entities with some level of demographic independence, mandating for conservation efforts (Ryder 1986, Moritz 1994), reviewed in Fraser and Bernatchez (2001).

Microsatellite markers suggest that the Baltic ringed seal population is only weakly differentiated from the Arctic seals, and may be connected to the oceanic seals by recurrent gene flow (*III*). Therefore, the genetic basis for the subspecies status of the Baltic population remains vague. Nevertheless, the inferred gene flow may not be large enough to prevent the evolution of adaptive differences, and recent history has shown that it is not enough to prevent population decline. This partial demographic independence, together with the distinctive distribution and history, warrants the treatment of Baltic ringed seals as an independent entity.

Although the origin of the mtDNA variation remains contentious, the uniqueness and conservation value of the Saimaa ringed seal population is indisputable. It is geographically, morphologically and ecologically distinct from the marine seals (Hyvärinen and Nieminen 1990). Isolated due to the postglacial geological changes, ringed seals of Lakes Saimaa and Ladoga represent survivors of perhaps several populations trapped in lakes and thus hold historical value (see Bowen 1999). Albeit unlikely to contribute greatly to biodiversity in the long

term, the Saimaa ringed seal is an important "umbrella species" for the conservation of the boreal lake environment. It is also a charismatic taxon, considered as one of the symbols of nature conservation in Finland.

The isolation history of the Saimaa ringed seals, as well as the recent decline and fragmentation of all postglacial populations, raises questions concerning the magnitude and consequences of loss of genetic diversity. Unless replaced by migration, erosion of genetic variation is an inevitable outcome of the enhanced drift in declined or fragmented populations (e.g. Nei 1987). Loss of variation may lead to lack of adaptive potential and lowered fitness i.e. inbreeding depression in small populations (e.g. Lynch and Walsh 1998).

It is generally assumed that the neutral genetic diversity revealed by DNA markers such as microsatellites and mtDNA control region sequences is positively correlated with levels of adaptive variation (Merilä and Crnocrak 2001, McKay and Latta 2002). This does not necessarily hold in all situations (see Table 2 in Hedrick 2001, see also Reed and Frankham 2001, Palo et al. 2003), however, unless there is a marked difference in the mutation rates between the neutral and adaptive loci, it is justified to assume a positive correlation in the Saimaa population. Preliminary results (Väinölä et al., *unpubl.*) suggest reduced diversity also in the DQB locus of the major histocompatibility complex (MHC) in the Saimaa ringed seals. MHC genes are involved in the immunological response and MHC variation is thus expected to be adaptive.

Järvinen and Varvio (1985) suggested that the Saimaa ringed seals would no longer be affected by inbreeding depression. Apart from a supposed postglacial purging of detrimental alleles, they assumed that even the initial genetic load was small, as very little allozyme variability had been found in marine seals (Simonsen et al. 1982). However, the notably high genetic variation in the marine ringed seals most likely also implies high levels of genetic load in the ancestral population. Purging of deleterious alleles is usually not strong enough to eliminate inbreeding depression completely (Ballou 1997). Furthermore, a progressive fitness decline due to accumulating deleterious mutations ("mutational meltdown") can constitute a substantial long-term extinction risk in populations with effective sizes even as high as 1000 (Lande 1995, Bürger and Lynch 1997, but see also Gilligan et al. 1997). Therefore, it seems unwarranted to suppose that past inbreeding would have effectively purged the population of genetic load and markedly reduced the risks from further inbreeding.

Increasing Saimaa ringed seal population size by reducing juvenile mortality is considered the best management strategy to counter demographic and genetic stochasticity (Kokko et al. 1998). Along with the population size increase, maintaining connectivity within the populations is important. Both the Saimaa and Baltic ringed seal populations have been recently fragmented, which could further increase the future rate of local inbreeding. Within the Baltic, this is most alarming in the Gulf of Finland population, holding only ca. 200 specimens.

However, from the microsatellite data, we estimated that this breeding area most likely remains connected to the other areas by gene flow (*III*). In contrast, in the Saimaa ringed seal the fragmentation into four separate breeding areas may adversely affect the population survival: Kokko et al. (1998) showed that decreasing the dispersal probability from 100% (no subdivision) to 25% increased the extinction risk tenfold. In genetic terms, we expect that reduced population densities would allow stronger differentiation and markedly enhance local inbreeding rates. Nevertheless, in both the Baltic Sea and Lake Saimaa, the threats from environmental and demographic stochasticity for future population survival are likely to remain greater than the genetic risks (Lande 1995).

4. CONCLUSION

From the late Pliocene onwards, the northern true seals have been subjected to the increased cyclicity of the northern hemisphere climate. These glacial periods have had impacts of varying degrees on the distribution, ecology and diversity of the extant species (King 1983).

Although the actual routes remain enigmatic, the continental invasion of now landlocked Caspian and Lake Baikal seals appears to coincide with the late Pliocene cooling ca. 2 Mya. It is also temporally associated with the radiation of the Phocina seal group, allegedly induced by the growing ice sheets in the northern Atlantic.

In the landlocked ringed seal populations included in this study, the molecular markers identify a two-fold impact of the glacial periods. In the Arctic Ocean ringed seals, the genetic diversity assessment suggests that the main ringed seal stock has been favoured by the extensive glaciations in the Middle and Late Pleistocene, contrary to many other taxa from the northern hemisphere. Most likely, the adaptation of ringed seals to the arctic conditions has enabled them to effectively exploit the increased area of pack ice during the glacial periods, allowing maintenance of large and connected populations throughout the Pleistocene. On the other hand, the marginal postglacially established ringed seal populations confined to the Fennoscandian basins show reduced intraspecific diversity. This has been documented in many species inhabiting previously glaciated areas and is caused by recurrent colonisation events, isolation and small long-term population sizes. The reduction of diversity has been particularly severe in the landlocked Lake Saimaa seal. Due to the combined effects of environmental, demographic and genetic stochasticity, this isolation history may critically affect the survival probability of this unique population.

6. REFERENCES

Allen, P. J., Amos, W., Pomeroy, P. P. and Twiss, S. D. 1995. Microsatellite variation in grey seals (*Halichoerus grypus)* shows evidence of genetic differentiation between two British breeding colonies. Molecular Ecology. 4: 653-662.

Andersen, B. G. and Borns Jr., H. W. 1997. The Ice Age World. Scandinavian University Press, Oslo, Norway.

Andersen, L. W., Born, E. W., Gjertz, I., Wiig, O., Holm, L. E. and Bendixen, C. 1998. Population structure and gene flow of the Atlantic walrus (*Odobenus rosmarus rosmarus)* in the eastern Atlantic Arctic based on mitochondrial DNA and microsatellite variation. Molecular Ecology. 7: 1323-1336.

Arbogast, B. S., Edwards, S. V., Wakeley, J., Beerli, P. and Slowinski, J. B. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annual Review of Ecology and Systematics. 33: 707-740.

Árnason, Ú., Bodin, K., Gullberg, A., Ledje, C. and Mouchaty, S. 1995. A molecular view of pinniped relationships with particular emphasis on the true seals. Journal of Molecular Evolution. 40: 78-85.

Árnason, Ú., Gullberg, A., Gretarsdottir, S., Ursing, B. and Janke, A. 2000. The mitochondrial genome of the sperm whale and a new molecular reference for estimating Eutherian divergence dates. Journal of Molecular Evolution. 50: 569-578.

Árnason, Ú., Xu, X., Gullberg, A.and Graur, D. 1996. The "*Phoca* Standard": An external molecular reference for calibrating recent evolutionary divergences. Journal of Molecular Evolution. 43: 41-45.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

Avise, J. C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conservation Biology. 9: 686-690.

Avise, J. C. 1996. Introduction: The scope of conservation genetics, p. 512. *In:* Conservation Genetics: Case histories from nature. J. C. Avise and J. L. Hamrick (eds.). Chapman & Hall, New York.

Avise, J. C. 1998. Conservation genetics in the marine realm. Journal of Heredity. 89: 377-382.

Avise, J. C. 2000. Phylogeography. Harvard University Press, Cambridge, MA.

Avise, J. C., Ankney, C. D. and Nelson, W. S. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics. 18: 489-522.

Avise, J. C., Walker, D. and Johns, G. C. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proceedings of the Royal Society of London Series B Biological Sciences. 265: 1707-1712.

Baker, C. S. and Palumbi, S. R. 1996. Population structure, molecular systematic and forensic identification of whales and dolphins. *In:* Conservation Genetics. Case histories from nature. J. C. Avice and J. L. Hamrick (eds.). Chapman & Hall, New York.

Ballard, J. W. O., Chernoff, B. and James, A. C. 2002. Divergence of mitochondrial DNA is not corroborated by nuclear DNA morphology, or behaviour in *Drosophila simulans*. Evolution. 563: .

Ballou, J. D. 1997. Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. Journal of Heredity. 88: 169-178.

Balloux, F. and Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology. 11: 155-165.

Berta, A. and Sumich, J. L. 1999. Marine Mammals: Evolutionary Biology. Academic Press, London.

Berta, A. and Wyss, A. R. 1994. Pinniped phylogeny. Proc. San Diego Soc. Nat. Hist. 29: 33-56.

Berthier, P., Beaumont, M. A., Cornuet, J. M., Luikart, G. 2002. Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: A

genealogical approach. Genetics. 160: 741-751.

Björck, S. and Sandgren, P. 1987. A Magnetostratigraphic comparison between ^{14}C years and varve years during the late Weichselian, indicating significant differences between the time-scales. Journal of Quaternary Science. 2: 133-140.

Bonner, J. 1994. Seals and Sea Lions (of the World). Blanford Press, London.

Boskovic, R., Kovacs, K. M., Hammill, M. O. and White, B. N. 1996. Geographic distribution of mitochondrial DNA haplotypes in grey seals (*Halichoerus grypus*). Canadian Journal of Zoology. 74: 1787-1796.

Bowen, B. W. 1999. Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. Molecular Ecology. 8: S5-S10.

Bowen, B. W., Bass, A. L., Rocha, L. A., Grant, W. S. and Robertson, D. R. 2001. Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. Evolution. 55: 1029-1039.

Bowen, B. W., Meylan, A. B., Ross, J. P., Limpus, C. J., Balazs, G. H. and Avise, J. C. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution. 46: 865-881.

Bremer, K. 1994. Branch support and tree stability. Cladistics. 10: 295-304.

Burg, T. M., Trites, A. W. and Smith, M. J. 1999. Mitochondrial and microsatellite DNA analysis of harbour seal population structure in the northeast Pacific Ocean. Canadian Journal of Zoology. 77: 930-943.

Burns, J. J. and Fay, F. H. 1970. Comparative morphology of the skull of the Ribbon seal, *Histriophoca fasciata*, with remarks on systematics of Phocidae. Journal of Zoology. 161: 363-394.

Bürger, R. and Lynch, M. 1997. Adaptation and extinction in changing environments, p. 209-239. *In:* Environmental Stress, Adaptation and Evolution. R. Bijlsma and V. Loeschke (eds.). Birkhäuser Verlag, Basel, Switzerland.

Caro, T. M. and Laurenson, M. K. 1994. Ecological and genetic factors in conservation: A cautionary tale. Science. 263: 485-486.

Chapman, M. R., Shackleton, N. J. and Duplessy, J.-C. 2000. Sea surface temperature varion during the last glacial-interglacial cycle: assessing the magnitude and pattern of climate change in the North Atlantic. Palaeogeography, Palaeoclimatology, Palaeoecology. 157: 1-25.

Chapskii, K.K., 1955a. Opyt peresmotra sistemy i diagnostiki tyulenei podsemeistva Phocinae. Trudy Zool. Inst. 47, 160-199.

Chapskii, K. K. 1955b. Contribution to the problem of the history and development of the Caspian and Baikal seals. Fish. Res. Board Can. Transl. Ser. 174: 1-26.

Comps, B., Gömöry, D., Letouzey, J., Thiébaut, T. and Petit, R. J. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European Beech. Genetics. 157: 389-397.

Davies, J. L. 1958. Pleistocene geography and the distribution of northern pinnipeds. Ecology. 39: 97-113.

De Muizon, C. 1982. Phocid phylogeny and dispersal. Annals of the South African Museum. 89: 175-213.

Demboski, J. R. and Cook, J. A. 2001. Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. Molecular Ecology. 10: 1227-1240.

Donner, J. 1995. The Quaternary History of Scandinavia. Cambridge University Press, Cambridge, UK.

Driscoll, C. A., Menotti-Raymond, M., Nelson, G., Goldstein, D. and O'Brien, S. J. 2002. Genomic Microsatellites as evolutionary chronometers: A test in wild cats. Genome Research. 12: 414-423.

Durant, S. M. and Harwood, J. 1986. The effects of hunting on Ringed seals (*Phoca hispida*) in Baltic. *In:* ICES c.m. Vol. 10.

Edwards, S. V. and Beerli, P. 2000. Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution. 54: 1839-1854.

Elverhøf, A., Dowdeswell, J. A., Funder, S., Mangerud, J. and Stein, R. 1998. Glacial and oceanic history of the Polar North Atlantic margins: an overview. Quaternary Science Reviews. 17: 1-10.

Emerson, B. C., Paradis, E. and Thébaud, C. 2001. Revealing the demographic histories of species using DNA sequences. Trends in Ecology and Evolution. 16: 707-716.

Engelbrecht, C. C., Freyhof, J. and Nolte, A. 2000. Phylogeography of the bullhead *Cottus gobio* (Pisces : Teleostei : Cottidae) suggests a pre-Pleistocene origin of the major central European populations. Molecular Ecology. 9: 709-722.

Eronen, M., Glückert, G., Hatakka, L., van der Plassche, O., van der Plicht, J. and Rantala, P. 2001. Rates of Holocene isostatic uplift and relative sea-level lowering of the Baltic in SW Finland based on studies of isolation contacts. BOREAS 30: 17-30.

Estoup, A. and Angers, B. 1998. Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. *In:* Advances in Molecular Ecology. G. R. Carvalho (ed.). IOS Press, Ansterdam, Netherlads.

Fedorov, V., Jaarola, M. and Fredga, K. 1996. Low mitochondrial DNA variation and recent colonization of Scandinavia by the wood lemming *Myopus schisticolor*. Molecular Ecology. 5: 577-581.

Forstén, A. and Alhonen, P. 1975. The Subfossil seals of Finland and their relation to the history of the Baltic Sea. Boreas. 4: 10-22.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: A review. Genetical Research. 66: 95-107.

Fraser, D. J. and Bernatchez, L. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology. 10: 2741-2752.

Frost, K. J. and Lowry, L. F. 1981. Ringed, Baikal and Caspian seals *Phoca hispida* Schreber, 1775; *Phoca sibirica*, Gmelin, 1788 and *Phoca caspica*, Gmelin, 1788., p. 29-53. *In:* Handbook of marine mammals vol. 2. Seals. S. H. Ridgway and R. J. Harrison (eds.). Academic Press, London.

Garza, J. C. and Williamson, E. G. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology. 10: 305-318.

Gemmell, N. J., Allen, P. J., Goodman, S. J. and Reed, J. Z. 1997. Interspecific microsatellite markers for the study of pinniped populations. Molecular Ecology. 6: 661-666.

Gilligan, D. M., Woodworth, L. M., Montgomery, M. E., Briscoe, D. A. and Frankham, R. 1997. Is mutation accumulation a threat to the survival of endangered populations? Conservation Biology. 11: 1235-1241.

Goldstein, D. B. and Schlötterer, C. 1998. Microsatellites: Evolution and Applications. Oxford University Press, Oxford, U.K.

Goodman, S. J. 1997. Dinucleotide repeat polymorphisms at seven anonymous microsatellite loci cloned from the European harbour seal (*Phoca vitulina vitulina*). Animal Genetics. 28: 310-311.

Goodman, S. J. 1998. Patterns of extensive genetic differentiation and variation among European Harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. Molecular Biology and Evolution. 15: 104-118.

Gottelli, D., Sillero Zubiri, C., Applebaum, G. D., Roy, M. S., Girman, D. J., Garcia Moreno, J., Ostranders, E. A. and Wayne, R. K. 1994. Molecular genetics of the most endangered canid: The Ethiopian wolf *Canis simensis*. Molecular Ecology. 3: 301-312.

Graves, J. E. 1998. Molecular insights into the population structures of cosmopolitan marine fishes. Journal of Heredity. 89: 427-437.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour. 28: 1140-1162.

Grigorescu, D. 1976. Paratethyan seals. Systematic Zoology. 25: 407-419.

Hartl, D. L. and Clark, A. C. 1997. Principles of Population Genetics. Sinauer associates Inc., Sunderland, Massachusetts.

Hasegawa, M., Kishino, H. and Yano, T. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution. 22: 160-174.

Hedrick, P. W. 2001. Conservation genetics: where are we now? Trends in Ecology and Evolution. 16: 629-636.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature. 405: 907-913.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence

and speciation. Biological Journal of the Linnean Society. 58: 247-276.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography - or seeing genes in space and time. Molecular Ecology. 10: 537-549.

Heyer, E., Zietkiexicz, E., Rochowski, A., Yotova, V., Puymirat, J. and Labuda, D. 2001. Phylogenetic and familial estimates of mitochondrial substitution rates: study of control region mutations in deep-rooting pedigrees. American Journal on Human Genetics. 69: 1113-1126.

Hillis, D. M., Moritz, C. and Mable, B. K. 1996. Molecular Systematics. Sinauer Associates, Inc., Sunderland, Massachusetts U.S.A.

Hoberg, E. P. 1995. Historical biogeography and modes of speciation across high-latitude seas of the Holarctic: concepts for host-parasite coevolution among the Phocini (Phocidae) and Tetrabothriidae (Eucestoda). Canadian Journal of Zoology. 73: 45-57.

Hoelzel, A. R., LeBoeuf, B. J., Campagna, C. and Reiter, J. 1999. Alpha male paternity in elephant seals. Behavioural Ecology and Sociobiology. 46: 298-306.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Oxford Survey of Evolutionary Biology 7: 1-44.

Hundertmark, K. J., Shields, G. F., Udina, I. G., Bowyer, R. T., Danilkin, A. A. and Schwartz, C. C. 2002. Mitochondrial phylogeography of moose (*Alces alces*): Late Pleistocene divergence and population expansion. Molecular Phylogenetics and Evolution. 22: 375-387.

Hyvärinen, H. and Nieminen, M. 1990. Differentiation of the ringed seal in the Baltic Sea, Lake Ladoga and Lake Saimaa. Finnish Game Research. 47: 21-27.

Hyvärinen, H. and Sipilä, T. 1992. Saimaannorppa *Phoca* (*Pusa*) *hispida saimensis*, p. 68-71. *In:* Maailman uhanalaiset eläimet. U. Elo (ed.). Weilin & Göös, Vantaa.

Hårding, K. and Härkönen, T. J. 1999. Developments of the Baltic grey seals (*Halichoerus grypus*) and ringed seal (*Phoca hispida*) populations during the 20th century. AMBIO. 28: 619-627.

Härkönen, T., Stenman, O., Iüssi, M., Iüssi, I., Sagitov, R. and Verevkin, M. 1998. Population size and distribution of the Baltic ringed seal (*Phoca hispida botnica*), p. 211-227. *In:* Ringed Seals in the North Atlantic. Vol. 1. M. P. Heide-Jörgensen and C. Lydersen (eds.). The North Atlantic Marine Mammal Commission, Tromsö, Norway.

Jarne, P. and Lagoda, P. J. L. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution. 11: 424-429.

Järvinen, O. and Varvio, S.-L. 1985. Proneness to extinction of small populations of seals: demographic and genetic stochasticity vs. environmental stress. Finnish Game Research. 44: 6-18.

Kapel, F. O., Christiansen, J., Heide-Jorgensen, M. P., Härkönen, T., Born, E. W., Knutsen, L. O., Riget, F. and Teilmann, J. 1998. Netting and conventional tagging used to study movements of ringed seals (*Phoca hispida*) in Greenland. NAMMCO Scientific Publications. 1: 211-227.

Kichler, K., Holder, M. T., Davis, S. K., Marquez, M. R. and Owens, D. W. 1999. Detection of multiple paternity in the Kemp's ridley sea turtle with limited sampling. Molecular Ecology. 8: 819-830.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution based on comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16: 111-120.

King, J. E. 1983. Seals of the world. British Museum and Oxford University Press, Oxford, UK.

Kishino, H. and Hasegawa, M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of Hominoidae. Journal of Molecular Evolution. 29: 170-179.

Kokko, H., Helle, E., Lindström, J., Ranta, E., Sipilä, T. and Courchamp, F. 1999. Backcasting population sizes of ringed and grey seals in the Baltic and Lake Saimaa during the 20th century. Annales Zoologici Fennici. 36: 65-73.

Kokko, H., Lindström, J. and Ranta, E. 1997. Risk analysis of hunting of seal popula-

tions in the Baltic. Conservation Biology. 11: 917-927.

Kokko, H., Lindström, J., Ranta, E., Sipilä, T. and Koskela, J. 1998. Estimating the demographic effective population size of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.). Animal Conservation. 1: 47-54.

Koretsky, I. 2001. Morphology and systematics of Miocene Phocinae (Mammalia: Carnivora) from Paratethys and the North Atlantic region. Geologica Hungarica, Series Paleontologica. Vol. 54.

Koretsky, I. A. and Holec, P. 2002. A primitive seal (Mammalia: Phocidae) from the early middle Miocene of Central Paratethys. Smithsonian Contributions to Paleobiology. 93: 163-178.

Koskela, J. T., Kunnasranta, M., Hämäläinen, E. and Hyvärinen, H. 2002. Movements and use of haul-out sites of radio-tagged Saimaa ringed seal (*Phoca hispida saimensis*) during the open water season. Annales Zoologici Fennici. 39: 59-67.

Kretzmann, M., Gemmell, N. J. and Meyer, A. 2001. Microsatellite analysis of population structure in the endangered Hawaiian Monk Seal. Conservation Biology. 15: 457-466.

Kunnasranta, M. 2001. Behavioural biology of two ringed seal (*Phoca hispida*) subspecies in the large European lakes Saimaa and Ladoga. University of Joensuu, Joensuu, Finland.

Kuusisto, E. 1999. Saimaa - a living lake, p. 205. Tammi, Helsinki.

Lande, R. 1988. Genetics and demography in biological conservation. Science. 241: 1455-1460.

Lande, R. 1995. Mutation and conservation. Conservation Biology. 9: 782-791.

LeDuc, R. G., Perrin, W. F. and Dizon, A. E. 1999. Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. Marine Mammal Science. 15: 619-648.

Li, W.-H. 1993. Unbiased estimates of the rates of synonymous and nonsynonymous substitution. Journal of Molecular Evolution. 36: 96-99.

Lopez, J. V., Culver, M., Stephens, J. C., Johnson, W. E. and O'Brien S. J. 1997. Rates of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals. Molecular Biology and Evolution. 14: 277-286.

Luikart, G. and England, P. R. 1999. Statistical analysis of microsatellite DNA data. Trends in Ecology and Evolution. 14: 253-256.

Lunt, D. H., Ibrahim, K. M. and Hewitt, G. M. 1998. mtDNA phylogeography and postglacial patterns of subdivision in the meadow grasshopper *Chorthippus parallelus*. Heredity. 80: 633-641.

Lynch, M. and Walsh, B. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, Massachusetts.

Macaulay, V., Richards, M. and Sykes, B. 1999. Mitochondrial DNA recombination - no need to panic. Proceedings of the Royal Society of London Series B Biological Sciences. 266: 2037-2039.

McKay, J. M. and Latta, R. G. 2002. Adaptive population divergence: markers, QTL, and traits. Trends in Ecology and Evolution. 17: 285-291.

McLaren, I. A. 1958. The Biology of the ringed seal (*Phoca hispida* Schreber) in the Eastern Canadian Arctic, p. 97. *In:* Bulletin. Fisheries Research Board of Canada, Ottawa, Canada.

McLaren, I. A. 1960. On the origin of the Caspian and Baikal seals and the paleoclimatological implications. American Journal of Science. 258: 47-65.

McLaren, I. A. 1966. Taxonomy of Harbour Seals of the western north Pacific and evolution of certain other hair seals. Journal of Mammalogy. 47: 466-473.

Merilä, J., Björklund, M. and Baker, A. J. 1997. Historical demography and present day population structure of the greenfinch, *Carduelis chloris* - an analysis of mtDNA control-region sequences. Evolution. 51: 946-956.

Merilä, J. and Crnocrak, P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology. 14: 892-903.

Moritz, C. 1994. Defining 'evolutionary significant units' for conservation. Trends in Ecology and Evolution. 9: 373-375.

Müller-Wille, L. 1969. Biometrical comparisons of four populations of *Phoca hispida* Schreb. in the Baltic and White Seas and lakes Ladoga and Saimaa. Commentationes Biologicae Societas Scientiarum Fennica. 31: 1-12.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nichols, R. A. 2001. Gene trees and species trees are not the same. Trends in Ecology and Evolution. 16: 358-364.

Nyakaana, S. and Arctander, P. 1999. Population genetic structure of the African elephant in Uganda based on variation at mitochondrial and nuclear loci: evidence for male-biased gene flow. Molecular Ecology. 8: 1105-1115.

O´Corry Crowe, G. M., Suydam, R. S., Rosenberg, A., Frost, K. J. and Dizon, A. E. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Molecular Ecology. 6: 955-970.

Palo, J. U., O'Hara, R. B., Laugen, A. T., Laurila, A., Primmer, C. R. and Merilä, J. 2003. Latitudinal divergence of common frog (*Rana temporaria*) life histroy traits by natural selection: evidence from a comparison of molecular and quantitative geneticdata. Molecular Ecology. 12: 1963-1978.

Pamilo, P. and Bianchi, N. O. 1993. The evolution of *Zfx* and *Zfy* genes: rates and interdependence between the genes. Molecular Biology and Evolution. 10: 271-281.

Pamilo, P. and Nei, M. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution. 5: 568-583.

Perry, E. A., Carr, S. M., Bartlett, S. E. and Davidson, W. S. 1995. A phylogenetic perspective on the evolution of reproductive behavior in pagophilic seals of the northwest Atlantic as indicated by mitochondrial sequences. Journal of Mammalogy. 76: 22-31.

Pesole, G., Gissi, C., De Chirico, A. and Saccone, C. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. Journal of Molecular Evolution. 48: 427-434.

Pirozhnikov, P. L. 1937. A Contribution to the study of the northern elements in the fauna of the Caspian Sea. Akad. Nauk Dokl. 15: 521-524.

Queller, D. C., Strassmann, J. E. and Hughes, C. R. 1993. Microsatellites and kinship. Trend in Ecology and Evolution. 8: 285-&.

Ranta, E., Lindström, J. and Kokko, H. 1996. Ecological risk analysis: The case of the Saimaa ringed seal. Ambio. 25: 363-365.

Ray, C. E. 1976a. Fossil marine mammals of Oregon. Systematic zoology. 25: 420-436.

Ray, C. E. 1976b. Geography of phocid evolution. Systematic Zoology. 25: 391-406.

Ray, N., Currat, M. and Excoffier, L. 2003. Intra-deme molecular diversity in spatially expanding populations. Molecular Biology and Evolution. 20: 76-86.

Reed, D. H. and Frankham, R. 2001. How closely related are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution. 55: 1095-1103.

Reeves, R. R. 1998. Distribution, abundance and biology of ringed seal (*Phoca hispida*): an overview, p. 9-45. *In:* Ringed Seals in the North Atlantic. Vol. 1. M. P. Heide-Jörgensen and C. Lydersen (eds.). The North Atlantic Marine Mammal Commission, Tromsö, Norway.

Rogers, A. R. and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution. 9: 552-569.

Ryder, O. A. 1986. Species Conservation and Systematics - the Dilemma of Subspecies. Trends in Ecology & Evolution. 1: 9-10.

Sage, R. D. and Wolff, J. O. 1986. Pleistocene glaciations, fluctuating ranges and low genetic variability in a large mammal. Evolution. 40: 1092-1095.

Salas, A., Lareu, V., Calafell, F., Bertranpetit, J. and Carracedo, A. 2000. mtDNA hypervariable region II (HVII) sequences in human evolution studies. European Journal of Human Genetics. 8: 964-974.

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Molecular Biology and Evolution. 14: 1218-1231.

Sasaki, H. and Numachi, K. I., 1997. The origin and genetic relationships of Baikal seal

elucidated by restriction analysis of mitochondrial DNA. In: Miyazaki, N. (Ed.), Animal community, environment, and phylogeny in Lake Baikal. Otsuchi Marine Research Center, Tokyo. pp. 91-105.

Scheffer, V. B. 1958. Seals, sea lions and walruses. Stanford University Press, Stanford.

Schneider, S. and Excoffier, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics. 152: 1079-1089.

Segerstråle, S. G. 1957. On the immigration of the glacial relicts of Northern Europe,with remarks on their prehistory. Commentationes Biologicae Societas Scientiarum Fennica. 16: 1-117.

Shimodaira, H. and Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution. 16: 1114-1116.

Simonsen, V., Allendorf, F. W., Eanes, W. F. and Kapel, F. O. 1982. Electrophoretic variation in large mammals. III. The ringed seal, *Pusa hispida*, the harp seal, *Pagophilus groenlandicus*, and the hooded seal, *Cystophora cristata*. Hereditas. 97: 87-90.

Sipilä, T., Helle, E. and Hyvärinen, H. 1990. Distribution, population size and reproductivity of the Saimaa ringed seal (*Phoca hispida saimensis* Nordq.) in Finland, 1980-84. Finnish Game Research. 47: 3-10.

Sipilä, T. and Hyvärinen, H. 1998. Status and biology of Saimaa (*Phoca hispida saimensis*) and Ladoga (*Phoca hispida ladogensis*) ringed seals. *In:* Ringed Seals in the North Atlantic. Vol. 1. M. P. Heide-Jorgensen and C. Lydersen (eds.). North Atlantic Marine Mammal Commission, Tromsö, Norway.

Sipilä, T. and Koskela, J. 2001. Saimaannorppien esiintymisalue keskittyy. Saimaan Luonto. 16: 5-7.

Sipilä, T., Medvedev, N. V. and Hyvärinen, H. 1996. The Ladoga seal (*Phoca hispida ladogensis* Nordq.). Hydrobiologia. 322: 193-198.

Smouse, P. E. 1998. To tree or not to tree. Molecular Ecology. 7: 399-412.

Springer, M. S., Murphy, W. J., Eizirik, E. and O'Brien, S. J. 2003. Placental mammal diversification and the Cretaceous-Tertiary boundary. Proceedings of the National Academy of Sciences. 100: 1056-1061.

Stanley, H. F., Casey, S., Carnahan, J. M., Goodman, S., Harwood, J. and Wayne, R. K. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). Molecular Biology and Evolution 13: 368-82.

Taberlet, P., Fumagalli, L., Cosson, A. G. and Wust-Saucy A. F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology. 7: 453-464.

Thiede, J., Winkler, A., Wolf-Welling, T., Eldholm, O., Myhre, A. M., Baumann, K.-H., Henrich, R. and Stein, R. 1998. Late Cenozoic history of the Polar North Atlantic: results from ocean drilling. Quaternary Science Reviews. 17: 185-208.

Väinölä, R. 1994. Molecular Systematics and Zoogeography of the "Glacial Relict" Element. *In:* Department of Genetic. University of Helsinki.

Zubakov, V. A. 2001. History and causes of variation in the Caspian Sea level: the Miopliocene, 7.1-1.95 Million years ago. Water Resources. 3: 249-256.